set forth above. Should such request or fee be deficient or absent, consider this paragraph such a request and authorization to withdraw the appropriate fee under 37 C.F.R. §§ 1.16 to 1.21 from Fulbright & Jaworski L.L.P. Deposit Account No. 50-1212/10102270/MBW.

Reconsideration of the application in view of the remarks contained herein is respectfully requested.

I. AMENDMENT

In the Claims:

Please cancel claims 55 and 74, without prejudice or disclaimer.

Please amend claims 54, 56, 57, 73, 75, 76, 80, 86 and 87 by replacing them with the following substitute claims:

54. (Amended) A method of treating an extract of a cell comprising:

(a) obtaining at least one cell;

(b) obtaining a reducing agent;

(c) preparing an admixture of an extract of the cell and the reducing agent; and

(d) heating the admixture.

56. (Amended) The method of claim 54, wherein preparing an admixture of an extract of the cell and the reducing agent comprises:

(a) first preparing an extract of the cell; and

(b) then mixing the extract with the reducing agent.

57. (Amended) The method of claim 54, wherein preparing an admixture of an extract of the cell and the reducing agent comprises:

(a) first mixing the cell and the reducing agent; and

(b) then preparing an extract of the cell in the presence of the reducing agent.

73. (Amended) A method for producing cDNA from one or more cells comprising:

(a) obtaining at least one cell;

(b) obtaining a reducing agent;

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(c) preparing an admixture of an extract of the cell and the reducing agent;

(d) heating the admixture; and

(e) incubating the admixture with reverse transcriptase under conditions to allow reverse transcription.

75. (Amended) The method of claim 73, wherein preparing an admixture of an extract of the cell and the reducing agent comprises:

(a) first preparing an extract of the cell; and

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(b) then mixing the extract with the reducing agent.

76. (Amended) The method of claim 73, wherein preparing an admixture of an extract of the cell and the reducing agent comprises:

(a) first mixing the cell and the reducing agent; and

(b) then preparing an extract of the cell in the presence of the reducing agent.

80. (Amended) A kit for producing cDNA from a cell, comprising, in one or more suitable containers:

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(a) a buffer; and

(b) a reducing agent.

86. (Amended) The kit of claim 80, further comprising a ribonuclease resistant RNA standard.

87. (Amended) A kit for producing cDNA from a cell comprising, in one or more suitable container(s):

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(a) a cell lysis buffer;

(b) a deoxyribonuclease;

(c) an RNase inhibitor;

(d) a reverse transcription buffer;

(e) reverse transcriptase;

(f) dNTPs;

(g) a reducing agent; and

(h) a ribonuclease resistant RNA standard.

Please add the following new claims

--88. (New) The method of claim 54, wherein the admixture is heated to a temperature that would result in the inactivation of RNase A, if any, present in the admixture.

89. (New) The method of claim 54, wherein the admixture is heated to a temperature that would result in the inactivation of RNase 1, if any, present in the admixture.

90. (New) The method of claim 54, wherein the admixture is heated to a temperature that would result in the inactivation of RNase T1, if any, present in the admixture..

91. (New) The method of claim 54, wherein the admixture initially comprises at least one ribonuclease which is inactivated by the combination of the reducing agent and heating.

92. (New) The method of claim 91, wherein the at least one ribonuclease is further defined as RNase A, RNase 1, or RNase T1.

93. (New) The method of claim 91, wherein the at least one ribonuclease is RNase A.

94. (New) The method of claim 91, wherein the at least one ribonuclease is RNase T1.

95. (New) The method of claim 91, wherein the at least one ribonuclease is RNase 1.

96. (New) The method of claim 54, wherein the admixture comprises at least one ribonuclease which was comprised in the cell and which is inactivated by the combination of the reducing agent and heating.

97. (New) The method of claim 54, wherein the reducing agent is a thiol-containing reducing agent.

98. (New) The method of claim 73, wherein the admixture initially comprises at least one ribonuclease which is inactivated by the combination of the reducing agent and heating.

99. (New) The method of claim 91, wherein the at least one ribonuclease is further defined as RNase A, RNase 1, or RNase T1.

100. (New) The method of claim 91, wherein the at least one ribonuclease is RNase A.

101. (New) The method of claim 91, wherein the at least one ribonuclease is RNase T1.

102. (New) The method of claim 91, wherein the at least one ribonuclease is RNase 1.

103. (New) The method of claim 91, wherein the admixture comprises at least one ribonuclease which was comprised in the cell and which is inactivated by the combination of the reducing agent and heating.

104. (New) The method of claim 79, wherein the reducing agent is DTT.

105. (New) The method of claim 104, wherein said the final concentration of the DTT is between 1 and 200 mM in the admixture.

106. (New) The method of claim 105, wherein the final concentration of DTT is 20 mM in the admixture.

107. (New) The method of claim 79, wherein said reducing agent is β-mercaptoethanol.

108. (New) The method of claim 107, wherein the final concentration of β-mercaptoethanol is between 1 and 200 mM in the admixture.

109. (New) The method of claim 79, wherein said reducing agent is cysteine.

110. (New) The method of claim 109, wherein the final concentration of cysteine is between 1 and 200 mM in the admixture.

111. (New) The method of claim 73, wherein the reducing agent is a thiol-containing reducing agent.

112. (New) The method of claim 73, wherein the reducing agent is comprised in a buffer composition prior to preparation of the admixture.

113. (New) The method of claim 73, wherein the admixture is heated to at least 37°C.

114. (New) The method of claim 113, wherein the admixture is heated to at least 60°C.

115. (New) The method of claim 73, wherein the admixture is heated to a temperature that would result in the inactivation of RNase A, if any, present in the admixture.

116. (New) The method of claim 73, wherein the admixture is heated to a temperature that would result in the inactivation of RNase 1, if any, present in the admixture.

117. (New) The method of claim 73, wherein the admixture is heated to a temperature that would result in the inactivation of RNase T1, if any, present in the admixture.

118. (New) The method of claim 73, wherein the admixture is heated for at least 4 minutes.

119. (New) The method of claim 87, wherein the reducing agent is comprised in the lysis buffer.

B7 cont. 120. (New) The method of claim 73, further defined as a method of preparing cDNA from a cellular extract without RNA purification.--

II. RESPONSE TO OFFICE ACTION

A. Status of the Claims

Claims 54-87 were pending at the time of the Action mailed on November 27, 2001. Claims 55 and 74 are cancelled herein without prejudice or disclaimer. Claims 54, 56, 57, 73, 75, 76, 80, 86, and 87 have been amended. Claims 88-120 have been added by amendment. Support for the amendment and newly added claims is found in the specification. That the specification supports claims 97 and 111 is indicated by the Action, at page 3. Therefore, claims 54, 56-73, and 75-120 are presently pending. A copy of the amended claims with editing indicia and the new claims is attached as Appendix A. A clean copy of all the presently pending claims as they will exist after entry of the amendments contained herein is attached as Appendix B.

B. Description of the Invention

The present invention relates to methods of treating cell extracts with a combination of a reducing agent and heat, as well as kits and combinations for carrying out such methods. These methods of treating cell extracts result in the inactivation of ribonucleases that may be present in the extract, thereby preventing degradation of any RNA that may be present in the cell.

Ribonucleases (RNases) are enzymes that process and degrade RNA molecules. In addition to the endogenous ribonucleases from cells and tissues, finger grease and bacteria and/or fungi in airborne dust particles are common sources of RNases in molecular biology procedures. Consequently, there is substantial potential for contamination of molecular biology samples by RNases of all forms. If present, RNases pose a significant risk of degrading RNA in numerous

procedures in the field of molecular biology. It is highly desirable to eliminate or minimize this risk.

The claimed methods have advantages over previously described methods of inactivating ribonucleases in cellular extracts that employ chaotropic agents, such as guanidinium-based compounds. Chaotropic agents can inhibit enzymes that might be used in subsequent molecular biology procedures. Therefore, RNA from cellular extracts treated with chaotropic agents must be removed from the presence of the chaotropic agent before it is employed in further enzyme-based molecular biological procedures. Such removal is usually accomplished by procedures involving washing, centrifugation to pellet the RNA, and then resuspension of the RNA in a solution that does not contain any of the chaotropic agent. Such procedures are time and labor consuming. By contrast, the presence of a reducing agent in a solution containing enzymes such as reverse transcriptase and/or DNase 1 does not result in the inhibition and/or inactivation of those enzymes. Therefore, there is no need to spend the time and labor to remove the reducing agent from the presence of the RNA in the methods of the invention.

In some specific embodiments, the invention relates to methods of preparing cDNA from cells. In such methods, the inventors have found that it is possible to employ the use of a reducing agent and heat to inactivate ribonucleases in a cellular extract and then employ the cellular extract in an appropriate protocol to produce cDNA from RNA present in the cellular extract. Further, because it is not necessary to remove the RNA from the reducing agent prior to adding enzymes such as reverse transcriptase and/or DNase 1, the methods of the invention allow for the preparation of cDNA in a single container, without laborious and time-consuming washing, centrifugation, and resuspension steps.

In addition to being useful to inactivate RNases that may be known to be present in a cellular extract, the claimed methods may be used prophylactically to eliminate the risk of contamination by RNases. This is important because a molecular biologist frequently does not know whether there are RNases present in his or her sample. It is, therefore, not necessary for a molecular biology sample to contain RNases in order for the methods of the claims to be useful in eliminating the risk of RNases. Further, it is not necessary for the methods of the invention to result in the inactivation of each and every type of ribonucleases that may exist for the methods to be of use to those skilled in molecular biology. The fact that RNases that are likely to be found in a cellular extract will be inactivated is sufficient to render the invention of use to the skilled practitioner. For example, treatment of a cellular extract in a manner proven to inactivate well-known ribonucleases, such as RNase A, RNase 1, and RNase T1 in order to remove the risk of RNase degradation from those ribonucleases is an important aspect of the invention.

C. The Non-statutory Double Patenting Rejection will be Overcome, as Appropriate, in Due Course

The Action rejects claims 54-57 and 61-72 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over copending application U.S. Serial No. 09/160,264 ("the '264 application"). The '264 application is currently on appeal.

If claims of the '264 application have been allowed or issued and the scope of the claims of that and the present application as of that time overlap, Applicants will prepare and file a Terminal Disclaimer, once the Examiner indicates that the presently pending claims are otherwise allowable.

D. The Rejection to Claims 54-60, 69-78, 80-83, and 85-87 Under 35 U.S.C §112, First Paragraph, is Overcome

The Action rejects claims 54-60, 69-78, 80-83, and 85-86 under 35 U.S.C §112, first paragraph, apparently stating that the full scope of the claim term "reducing agent" is not described and/or enabled by the specification. The Action suggests that the term "thiol-containing reducing agent" comports with the requirements of 35 U.S.C §112, first paragraph. Applicants traverse this rejection, because there is adequate written description and enablement of the term "reducing agent" in the specification.

As an initial point, the Action wholly fails to meet the burden upon the Patent and Trademark Office to make either a written description rejection or an enablement rejection. As set forth in M.P.E.P. 2163.04, "a description as filed is presumed to be adequate, unless or until sufficient evidence or reasoning to the contrary has been presented by the examiner to rebut the presumption. Therefore, in order to meet the burden upon him with regard to a written description rejection, "The examiner has the initial burden of presenting by a preponderance of evidence why a person skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims." *Id.* Likewise, as set forth in M.P.E.P. 2164.04, a specification "must be taken as being in compliance with the enablement requirement of 35 U.S.C. §112, first paragraph, unless there is a reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support." In regard to an enablement rejection, "it is incumbent upon the Patent Office...to explain *why* it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement." *Id.*, citing *In re Marzocchi*, 439 F.2d, 220, 224, 169 U.S.P.Q. 367, 370 (CCPA 1971) (emphasis in original).

The Action fails to provide any evidence or reasoning as to why the term "reducing agent" lacks adequate written description in the specification and/or is not enabled in the specification Rather, the total substance of the rejection, as found at page 3 of the Action, is:

> In the instant disclosure and in claims not listed in this rejection (e.g. claims 61-68) applicant has listed specific reducing agents limited to thiol (-SH) containing compounds and in independent claims has relied on the term "reducing agent." The compounds which are not thiol containing agents but are "reducing agents" and which are not enabled herein is substantial. Therefore, the instant written description fails to provide adequate support for the breadth of claims wherein the non-thiol reducing agents are included. Limitation to thiol containing reducing agents is respectfully requested.

This statement, contrary to the requirements of M.P.E.P. 2163.04 and 2164.04 and the relevant rules and case law, contains absolutely no explanation as to why one of ordinary skill in the art would doubt the written description and/or enablement of the claims. The Action certainly provides no evidence or reasoning upon which one could base any rejection under 35 U.S.C. §112, first paragraph, under a preponderance of the evidence. For this reason alone, this rejection must be withdrawn.

Further, the present claims fully comply with the written description and enablement requirements of 35 U.S.C. §112, first paragraph.

Regarding the written description requirement, the term "reducing agent" is found throughout the specification and originally filed claims, and has a definition that is well-understood to those of skill in the art to include a variety of compounds, including thiol-containing and non-thiol-containing compounds. Applicants set forth adequate written description for this term at page 6, lines 20-22, where they state, "Many agents having reducing properties are well known to those of skill in the art, and, by employing assays described herein, one of ordinary skill in the art will be able to determine which of any of these *reducing agents* will be of use in the present invention." (Emphasis added). The specification then details

specific preferred reducing agents. In fact, it is clear from the portion of the Action cited above that the Examiner recognizes this definition of the broad scope of this term. Therefore, contrary to the statements of the Action, the written description requirement is met by the specification and claims.

Regarding the enablement requirement, the full scope of the present claims of the specification is enabled by the specification, which: 1) describes the use of "reducing agents" in the context of the invention; 2) provides a definition of that term, as cited above; 3) sets forth specific preferred agents; 4) details studies proving the use of such reducing agents in practice; and provides assays and protocols that will allow one of ordinary skill in the art to determine which of any reducing agents will be of use.

Dr. Brent Iverson has provided a Declaration, attached as Appendix C, which supports the enablement of the present claims ("the Iverson Declaration").

Dr. Iverson is an Associate Professor at the University of Texas at Austin in the Department of Chemistry and Biochemistry, and a member of the Institute of Cellular and Molecular Biology, also at the University. He has authored numerous publications in the field of molecular biology and has received a host of academic, research and teaching awards. Dr. Iverson's current research involves antibody and enzyme engineering, and study of the chemistry of nucleic acid binding, recognition and modification. Iverson Declaration, paragraph 1. Dr. Iverson understands the invention, has reviewed relevant materials to allow him to make a declaration in regard to this application, and understands the substance of the instant rejection. Iverson Declaration, paragraphs 2-4.

According to Dr. Iverson:

> The specification clearly sets forth that many agents and reducing agents are known to those of skill, see, for example, page 6, lines 20-23. The specification also sets forth assays that can be used by one of ordinary skill in the art to determine, without undue experimentation, whether or not any given reducing agent will work in the context of the invention. For example, such assays are described in Example 1 of the specification, at page 12, line 6 to page 13, line 26.

Iverson Declaration, paragraph 5.

Dr. Iverson concludes that "a skilled molecular biologist will be able to practice the claimed invention with any reducing agent that will function in the context of the invention, including, but not limited to, 'thiol containing reducing agents.'" Iverson Declaration, paragraph 6.

In view of the above, the rejection to claims 54-60, 69-78, 80-83, and 85-86 under 35 U.S.C. §112, first paragraph is overcome.

E. The Rejection to Claim 87 Under 35 U.S.C §112, First Paragraph, is Overcome

The Action rejected claim 87 under 35 U.S.C §112, first paragraph, evidently because that claim did not recite any "reducing agent." Present claim 87 does specifically recite that "a reducing agent" is comprised in the claimed kit. Therefore, this rejection is overcome. Further, the term "reducing agent" as employed in current claim 87 has adequate written description and enablement in the specification, for the reasons set forth above.

F. The Rejection to Claims 54-79 Under 35 U.S.C. §112, First Paragraph, is Overcome

The Action rejects claims 54-87 Under 35 U.S.C. §112, first paragraph. Although the Action is not clear in regard to the exact nature of the rejection, it appears to Applicants that this is a scope of enablement rejection, in that the Action seeks to limit the scope of the claims to the

specific embodiments. Applicants traverse because the instant claims are fully enabled and have full written support in the specification.

Applicants believe that the Examiner is basing this rejection upon a belief that some enzymes or compounds that might be classified as "ribonucleases," in that they degrade RNA, might not be inactivated by the use of a reducing agent and heat, as described in the present specification and claimed in the present claims. That appears as if this is the gist of the arguments based on Murthy *et al.* and Khesin *et al.* Applicants note that similar rejections are pending in the aforementioned '264 application, which is on appeal from Examiner Crane at this time.

1. The Specification Fully Enables the Claims.

The Action appears to find theoretical reasons from various pieces of art as to why the invention might not work and bases the instant rejection on those pieces of art. As will be discussed in detail below, these pieces of art do not, in fact, suggest any lack of enablement of the instant claims and are irrelevant to the instant invention. However, even if these references did suggest a theoretical reason why there might be an issue with the claims, which Applicants dispute, the actual data in the specification supports that the present claims are fully enabled and have written description.

For example, Examples 12, 13, and 14 of the specification, which are found at page 21, line 14, to page 27, line 22, set forth studies that show, despite the suggestions of the Action, that the invention functions to inactivate ribonucleases found in cell extracts, at least to a level that prevents degradation of the RNA contained in the cell extracts, until cDNA can be produced from the extract. These data show that the enablement of the invention, and prove that, in the real world, the theoretic concerns raised by the Action regarding, for example, "inhibitor induced

RNAse insensitivity," and that "RNAse 'BS-1' retains some or all of its enzymatic activity after reduction of the RNAase with DTT at 37°C either in the presence of an RNAse inhibitor or in the absence thereof" do not prevent the functioning of the claimed invention.

2. Murthy *et al.* Does Not Relate to Inactivation of Ribonucleases with Reducing Agents.

Despite the suggestion of the Action, the Murthy *et al.* reference in no way discloses the use of a reducing agent to inactivate ribonucleases, or fail to inactivate ribonucleases. Rather, the reference provides information on the use of an entirely different agent (human placental RNase inhibitor "hPRI") for that purpose.

In regard to Murthy *et al.*, the Action appears to find that Murthy *et al.* suggests that it is possible for RNA to interfere with the action of the reducing agents employed in the invention and thereby render the reducing agents ineffective in inactivating RNases. Based on this theoretical suggestion, the Action states:

> Because the instant disclosure does not appear to present a clear and cogent explanation of how applicant's (sic) have overcome the problem of inhibitor induced RNAse insensitivity to a thiol reducing agent disclosed in the noted prior art disclosure, examiner concludes that the instant claims are at a minmum lacking in enablement for exemplifications wherein as noted in Murthy et al. the inhibitor has no effect on the enzyme because of the interaction of the enzyme with the RNA substrate.

Action, page 4. Further, the Examiner appears to suggest that the Murthy reference discloses that it is possible for some ribonucleases, but not all ribonucleases, to be inactivated by the methods of the invention.

As an initial point, Applicants point out that, as shown in the specification, these theoretical issues raised by the Action do not play out in real life. Further, Applicants must point out that the Action appears to attempt to shift the burden of proving enablement to Applicants, in contravention of the requirements of the law. Applicants have absolutely no burden or

requirement to explain why their invention works, so long as they can demonstrate that their invention does work, which they have done in the specification.

i. Murthy et al. does not teach inhibition of the ribonuclease inhibiting ability of reducing agents by RNA.

Murthy *et al.* does not demonstrate that there is any interaction between reducing agents, such as DTT, and RNA that would prevent such reducing agents from functioning to inactivate RNases when employed in the context of the invention. As such, the Action, even with its citation of Murthy *et al.*, fails to provide the evidence required under M.P.E.P. 2164.04.

This rejection appears to be based on a misreading of the Murthy *et al.* reference by the Examiner. The Murthy *et al.* reference relates to the inhibition of three ribonucleases by human placental RNase inhibitor ("hPRI"), *not* by a reducing agent. Murthy *et al.* does not disclose treating samples containing a ribonuclease with a reducing agent and heat for the attempted purpose of eliminating ribonuclease activity at all. Rather, Murthy *et al.* discloses the use of hPRI for that purpose. It appears to Applicants that the confusion of the hPRI employed by Murthy *et al.*, which is refered to as the "inhibitor" in that paper, with DTT, which is not ever refered to as an "inhibitor" in the paper, is a the root of this rejection.

Contrary to the Examiner's apparent conclusion that Murthy *et al.* employed DTT to inactivate RNase A and RNase BS-1, one of ordinary skill in the art would recognize that DTT was added to the reaction mixture in the study not as an RNase inhibitor, but rather because hPRI *requires* DTT in order to function. *See*, for example, Blackburn *et al.*, "Ribonuclease inhibitor from human placenta. Purification and properties," *J. Biol. Chem.* 252(16):5904-5910, 5907 (1977), attached as Appendix D, which states "The loss of inhibitor activity was related to the loss of DTT from the initial extract." The present claims relate to methods in which ribonucleases that may be present in a cell sample are inactivated by a reducing agent. Unlike

Murthy *et al.*, these claims do not require use of hPRI. Whether the hPRI used by Murthy *et al.* was effective in eliminating ribonuclease activity is irrelevant to the claims of the present application.

Murthy *et al.* did not employ, or attempt to employ, DTT or any other reducing agent in combination with heat to inactivate a ribonuclease. While Murthy *et al.* may evidence that hPRI was not able to inactivate all three of the ribonucleases tested under the specific conditions employed therein, such data do not address the operability of reducing agents in inactivating ribonucleases with the methods claimed in the instant application.

Applicants have presented data in the Specification that amply demonstrates the efficacy of reducing agents in inactivating a variety of known and unknown ribonucleases according to the inventors' methods. Specification, page 14, line 20, to page 21, line 12, and page 27, lines 24-30. In view of this, the present claims are enabled, despite the Examiner's interpretation of Murthy *et al.*

ii. There is no need to limit the claims with regard to specific ribonucleases inactivated.

The Action has requested that that Applicants limit the scope of their claims to embodiments which are enabled. Applicants do not fully understand the Action's request in this regard and assert that, regardless, the entire scope of the present claims is enabled. However, it appears, based on the Action, that the Examiner is seeking limitation of the claims to the inactivation of only a few, specific ribonucleases.

The Action is incorrect in requesting limitation of the claims in at least four manners. First, the specification contains examples demonstrating the use of the combination of a reducing agent and heat to accomplish the goals of the invention by inactivating ribonucleases in cell extracts, thereby proving that whatever theoretical presumptions the Action has surmised from

Murthy *et al.* or any other art are incorrect. Second, Murthy *et al.*, as properly read by one of ordinary skill in the art, does not teach that the conditions of the present claims, involving the use of a reducing agent and heat, is insufficient to inactivate any ribonuclease, and Dr. Iverson's Declaration sets forth facts supporting that this is the case. Third, even if there were some ribonucleases which were not inactivated by the claimed conditions, which Applicants do not admit and the Action has not shown, as a matter of law, claims need not exclude any and all inoperative embodiments. Finally, under the law, as supported by facts set forth in the Declaration of Dr. Iverson, the present claims would not require undue experimentation to practice.

Examples 12-14 of the specification clearly demonstrate the enablement of the presently claimed invention, in that they present evidence of the use of the combination of a reducing agent and heat to protect the RNA in cellular extracts, at least to the extent to allow isolation of full length RNA from a cellular extract and the preparation of cDNA from a cellular extract.

To the extent that the Examiner appears to be premised on the indication in Murthy *et al.* that RNase BS-1 was not sensitive to hPRI at low levels under the conditions employed in the study, Applicants reiterate that Murthy *et al.*, regardless of what it says about the use of hPRI to inactivate RNase BS-1, does not speak to the use of or ability of the combination of a reducing agent and heat, as set forth in the present claims, to do so. The Action provides no evidence to suggest that a combination of a reducing agent and heat according to the invention would not inactivate RNase BS-1.

While the claim scope that the Examiner considers enabled is not apparent from the Examiner's request, Applicants believe the Examiner intends that the claims be limited to cover inactivation of only those ribonucleases that are specifically exemplified in the Specification.

However, in view of the data provided in the Specification at page 16, line 6, to page 13, line 26 and page 14, line 20, to page 28, line 20, one of ordinary skill in the art would conclude that the claimed methods would be effective to inactivate ribonucleases in addition to those exemplified present in a cell extract. The Iverson Declaration supports this contention. As stated in the Dr. Iverson's Declaration, "[a] skilled molecular biologist will readily apply the methods disclosed in the specification...to inactivate ribonucleases including, but not limited to, RNase A, RNase 1, and RNase T1...That the disclosed methods can be used to inactivate ribonucleases in general...is apparent from the specification." Appendix C, paragraphs 7-9.

Further, even if the exemplified and claimed invention employing the combination of a reducing agent and heat were not sufficient to inactivate all possible ribonucleases, which Applicants do not admit and the Examiner has not shown, the claims would not be properly rejectable under 35 U.S.C. §112, first paragraph. "It is not a function of the claims to specifically exclude either possible inoperative substances or ineffective reactant proportions." *In re Dinh-Nguyen and Stenhagen,* 492 F.2d 856, (C.C.P.A. 1974); see also *In re Hradcovsky,* 214 U.S.P.Q. 554 (PTO Bd. App. 1982) and *Atlas Powder Co. v. E. I. du Pont de Nemours & Co.,* 588 F.Supp. 1455 (Tex. 1983). Applicants are not required to exclude from the claims every possible ribonuclease on which the methods may be ineffective.

Furthermore, enablement is not precluded by the necessity for some experimentation, as long as the amount of experimentation is not unduly extensive. *In re Wands,* 858 F.2d 731 (Fed.Cir. 1988); *Hybritech Inc. v. Monoclonal Antibodies, Inc.,* 802 F.2d 1367 (Fed.Cir. 1986); *Atlas Powder Co. v. E.I. du Pont de Nemours & Co.,* 750 F.2d 1569 (Fed.Cir. 1984) ("That some experimentation is necessary does not constitute a lack of enablement..."). Under these standards, unduly extensive experimentation by a skilled artisan would not be required in order

to make and use the invention commensurate with the scope of the claims, even if there were some ribonuclease that the Examiner could point to which was not inactivated by the methods or compositions of the present claims. According to Dr. Iverson "the specification teaches how to test for inactivation of other ribonucleases [in addition to RNase A, RNase 1, and RNase T1] at page 12, line 6, to page 14, line 26." Appendix C, paragraph 10. It is matter of established case law that any and all inoperative embodiments of the invention need not be excluded from the claims. *In re Dinh-Nguyen and Stenhagen,* 492 F.2d at 859; *Atlas Powder Co. v. E. I. du Pont de Nemours & Co.,* 588 F.Supp. at 1469. As confirmed by Dr. Iverson, "determining whether the methods disclosed in the specification are effective to inactivate any particular given ribonuclease requires merely routine experimental work by a laboratory technician trained in standard techniques." Appendix C, paragraph 10.

iii. Khesin et al. does not demonstrate any fact of relevance to the patentablility of the claims.

The Action cites Khesin *et al.* in an attempt to bolster its argument regarding lack of enablement. Specifically, the Action states that Khesin *et al.*:

> Directly contradicts the assumption that the action of reducing agents and the like on ribonucleases always reduces ribonuclease activity, suggesting that limitations on claim scope are essential in order to take this reference into account.

Applicants do not fully understand what the Action is attempting to convey in this regard, primarily, because the Action does not meet the burden placed on the Patent Office according to M.P.E.P 2164.04 of providing facts and reasoning to support the rejection. This statement is devoid of any alleged facts or reasoning.

Upon review of the full reference, Applicants see nothing in Khesin *et al.* that is of any relevance to the present claims. To the extent that the Action is attempting to use Khesin *et al.* to bolster its argument that there may be some ribonucleases that are not inactivated by the

combination of a reducing agent and heat claimed in the present claims, the Action does not explain where it finds any support for this proposition in Khesin *et al.* Applicants see nothing in the Khesin *et al.* reference that would support such a proposition. Further, as set forth above, even if Khesin *et al.* did set forth that there were some ribonuclease that would not be inactivated by the methods of the present invention, this would not render the present claims unpatentable under 35 U.S.C. §112, first paragraph.

In view of the above, Applicants submit that all of the pending claims are operative and enabled, satisfying the requirements of 35 U.S.C. § 112, first paragraph, and respectfully request that the rejection in this regard be withdrawn.

G. The Rejections Under 35 U.S.C. §112, Second Paragraph, are Overcome

The Action entered a variety of rejections against various of the claims based on 35 U.S.C. §112, second paragraph. For reasons set forth below, these rejections are overcome.

1. Current Claim 54 is Definite.

The Action rejected claim 54 as indefinite, stating that it claimed a method without setting forth what the method was a method of doing. While Applicants disagree with the Action's statements in this regard, in order to progress the allowance of claims, current claim 54 has been amended to recite that it is directed to "A method of treating an extract of a cell." Therefore, the claim is definite and the rejection is overcome.

2. The Rejection to Claims 55 and 74 is Overcome.

The Action rejected claims 55 and 74, stating that the phrase "is further defined as comprising" was indefinite. Applicants disagree that this language was indefinite. However, amendment to the claims has caused claims 55 and 74 to be cancelled without prejudice or

disclaimer. Therefore, this rejection is moot. The Action also states, "Similar problems also occur in claims 56-58, 74-76 and 81." Applicants do not find the phrase "is further defined as comprising" in any of these claims. Further, Applicant's review of those claims indicates that they are definite. Applicnats are happy to entertain any suggestions from the Examiner in this regard.

3. The Rejection to Claim 81 is Overcome.

Claim 80 has been amended such that it is now clear that claim 81 further limits the subject matter of claim 80.

4. The Rejection to Claims 86 and 87 is Overcome.

The Action rejected claims 86 and 87 for their recitation of the Ambion trademark "Armored RNA®." The claims have been amended to recite the generic term "ribonuclease resistant RNA standard, which is supported by the specification and understood by those of skill in the art. Therefore, this rejection is overcome.

H. Murthy *et al.* Teaches Nothing about the Use of a Reducing Agent and Heat to Inactivate Ribonulceases

Turning to the rejections over the art, Applicants note that the primary reference leveled at the claims is that of Murthy *et al.* In fact, the majority of the references appear premised upon a belief that Murthy *et al.* teaches the use of a reducing agent to inactivate ribonucleases. This is not the case.

It is not taught in Murthy *et al.* that, under the conditions of the claims, reducing agents, including DTT, inhibit ribonucleases. Murthy *et al.* does not disclose inactivation of ribonucleases by a combination of a reducing agent and heat. Murthy *et al.* does not disclose treating samples containing a ribonuclease with a reducing agent for the purpose of eliminating

ribonuclease activity at all. Rather, Murthy *et al.* discloses the use of hPRI for inhibiting the ribonucleases. Unlike the inactivation methods of Murthy *et al.*, the present claims do not require use of hPRI. Whether the hPRI used by Murthy *et al.* was effective in eliminating ribonuclease activity is irrelevant to the claims of the present application.

Murthy *et al.* may suggest the use of DTT to monomerize a specific RNase, which is typically found in a dimeric form, in studies to elucidate the effects of hPRI (a ribonuclease inhibitor that is not a reducing agent) on that RNase. However, this is of no relevance to the present claims. In contrast to Murthy *et al.*, all of the presently claimed methods involve methods that allow for inactivation of ribonucleases by a combination of a reducing agent and heat, not by hPRI.

Murthy *et al.* studied the sensitivity of different forms of RNase BS-1 to hPRI under different experimental conditions. The forms of RNase BS-1 studied included the native form, (dimeric), MCM RNase BS-1 (the purified stable monomeric form), and RNase BS-1 pretreated with 5 mM DTT (a mixture of dimeric and monomeric forms under the experimental conditions used). For comparison, the sensitivity of bovine pancreatic RNase (RNase A) to hPRI under the experimental conditions was also determined. Assays for the ribonucleases studied were carried out in the presence of a buffer with 5 mM DTT and 1 mM DTT. The authors concluded that the monomeric form (MCM RNase BS-1) and RNase A exhibited comparable sensitivities to hPRI. The dimeric form of RNase BS-1 was considered to be insensitive to hPRI unless pretreated with DTT, which converted the dimer into monomers.

The Examiner has specifically directed Applicants to Fig. 1 of the reference. However, that figure shows the percent of inhibition of three ribonucleases (RNase A, MCM-RNase BS-1 and RNase BS-1) by hPRI, *not* by DTT (a reducing agent). The assays were run in the presence

of a buffer with 5 mM DTT. As discussed above, it is well known in the art that hPRI requires DTT in order to function. *See*, for example, Blackburn *et al.*, Appendix D. Therefore, the presence of DTT in the buffer employed to study hPRI would be expected and is not indicative of a teaching of the present invention. In marked contrast to the Examiner's interpretation of Fig. 1 of Murthy *et al.*, the figure clearly shows that, when the assays were carried out in the presence of DTT without hPRI (amount of inhibitor = 0), there was no inhibition of the ribonucleases (inhibition % = 0) for all three ribonucleases studied. Fig. 3 confirms the results for RNase BS-1.

Not only does this reference fail to disclose inactivation of ribonucleases by reducing agents, one of ordinary skill in the art reading this reference would conclude, as has Dr. Iverson, that "contrary to the examiner's interpretation, one of skill in the art, upon reading the Murthy *et al.* reference, without the benefit of the teachings of the present application and inventors, would believe that DTT cannot be used to inactivate ribonucleases." Iverson Declaration, paragraph 14, Appendix C. Of course, Applicants have shown that it is possible to use a combination of a reducing agent and heat to inactivate ribonucleases. However, this is not taught in Murthy *et al.*, and the attempt to find such a suggestion in Murthy *et al.* is impermissible hindsight reconstruction.

Further, the data in Figs. 1-4 of Murthy *et al.* reveal that the claimed limitation (treatment of a cellular extract with the combination of a reducing agent) is not inherent or otherwise implicit in the reference. Nothing in the reference would lead one of ordinary skill to anticipate successful inactivation of ribonucleases by a reducing agent. Dr. Iverson points out that the reference, in fact, reflects the contrary, in that the data provided in the Murthy *et al.* reference

would indicate that DTT alone was not sufficient to inactivate ribonucleases in the conditions employed in the reference. *See* Iverson Declaration, paragraph 15, Appendix C.

I. Murthy *et al.* Does Not Anticipate Claims 80-85.

The Examiner rejects claims 80-85, which are all directed to kit, as anticipated by Murthy *et al.* The rejection is not warranted by this reference.

Anticipation requires identity of invention. For a prior art reference to anticipate, every element of the claimed invention must be identically shown in a single reference. *In re Bond,* 910 F.2d 831 (Fed.Cir. 1990). Murthy *et al.* contains no mention of a kit for the production of cDNA anywhere in its disclosure. Further, Murthy *et al.*, does not mention the preparation of cDNA anywhere in its disclosure. Therefore, this reference cannot anticipate any of claims 80-85. Particularly, Murthy *et al.* cannot anticipate claims 82 and 84, which respectively recite that the kit comprises reverse transcriptase and/or a deoxyribonuclease. Applicants find nowhere in the disclosure of Murthy *et al.* where the inclusion of these enzymes, in any reaction, much less a kit, is taught. Therefore, the anticipation rejection over Murthy *et al.* is overcome.

Further, as set forth above, Murthy *et al.* does not teach or suggest the use of DTT, or any other reducing agent, as compounds that, in combination with heat, inactivate ribonucleases. Therefore, to the extent that the Action premises this rejection upon such a reading of Murthy *et al*, this rejection is overcome.

As a final point, Applicants do not understand the Action's comments at page 7 concerning "claim 31" and the suggestion that both "acetate" and "DTT" are "chelators." Claim 31 is not pending in the present case and the term "chelator(s)" does not appear in the pending claims. Therefore, Applicants decline to note any relationship or lack thereof of "acetate" and "DTT" as "chelators."

In view of the above, Applicants respectfully request that rejection under 35 U.S.C. § 102(b) be withdrawn.

J. Claims 54-57 and 61-72 Are Not Obvious Over the Combination of Murthy *et al.* in View of Boshes and in Further View of Cleland

Claims 54-57 and 61-72 are rejected by the Action under 35 U.S.C. § 103(a) as obvious. Applicants respectfully traverse these rejections.

1. The Murthy *et al.* Reference Provides No Support for an Obviousness Rejection.

The Murthy *et al.* reference provides no suggestion or motivation to combine its teachings with other art to practice the claimed methods. More significantly, as set forth above, the reference suggests that DTT could not be used to inactivate ribonucleases under the testing conditions used in the reference. Therefore, the reference provides no support for an obviousness rejection.

Because Murthy *et al.* does not teach the use of <u>any</u> reducing agent to inactivate ribonucleases, it cannot provide a basis for the claims to be held obvious in the manner suggested by the Examiner. "The Murthy *et al.* references does not teach or suggest the use of DTT or any other reducing agent to inactivate ribonucleases. Furthermore, the Murthy *et al.* reference does not provide any motivation to use DTT or any other reducing agent to inactivate ribonucleases or suggest any likelihood of success in doing so. Indeed, Murthy *et al.* indicates that neither DTT nor other reducing agents can be used to inactivate ribonucleases under the conditions disclosed in it, and this would have suggested to skilled molecular biologists without the benefit of the teachings of the present application that the methods claims in the present application would not succeed." Iverson Declaration, paragraph 15, Appendix C.

As the Iverson Declaration shows, Murthy *et al.* does not provide to one of skill in the art the suggestion or motivation that he or she should attempt to carry out the claimed methods, and certainly does not provide any reasonable expectation to one of ordinary skill in the art that he or she could succeed in using reducing agents to inactivate nucleases. In fact, the reference suggests that there would be no reasonable expectation of success for the present invention, by showing that reducing agents were not able to inactivate ribonucleases. For example, in Figs. 1-4 of the reference, it was shown that under the conditions employed therein, DTT was not able to inactivate the ribonucleases tested. In this regard, Murthy *et al.* teaches away from the invention. Dr. Iverson's declaration states that "a skilled molecular biologist would be actively dissuaded from using DTT or any other reducing agent to inactivate ribonucleases, based on the information provided in Murthy *et al.*" Iverson Declaration, paragraph 16, Appendix C. "Inventing a method for producing an effective product, in the face of art which strongly suggests that such a method would produce unacceptable results, is the very antithesis of obviousness." *In re Rosenberger and Brandt,* 386 F.2d 1015 (C.C.P.A. 1967). Applicants proceeded in a manner contrary to that suggested by Murthy *et al.*, and discovered the claimed methods.

2. Boshes Does Not Support Inactivation of Ribonucleases with a Combinatin of a Reducing Agent and Heat.

The Boshes reference, which was discussed at length in the Specification, does not support the use of reducing agents to inactivate ribonucleases. *See* Boshes *et al.*, *J. Cell Bio.* 46:477-490 (1970). As set forth in the Specification, Boshes describes an experiment, in which polyribosome preparations were treated with RNase A in a solution in the presence or absence of 4 mM DTT. The treatment of polyribosomes with RNase A generated monoribosomes. Boshes observed that polyribosomes treated with RNase A in the presence of 4 mM DTT decreased the

conversion of polyribosomes to monoribosomes. Since Boshes was working with a complex, uncharacterized protein mixture, it is unclear what may have been responsible for the decreased production of monoribosomes reported in the paper. Boshes did not report any assay of RNase A activity, with or without exposure to DTT. The effect of DTT on the conversion of a complex mixture of polyribosomes to monoribosomes by RNase A would not suggest to one of ordinary skill that DTT, or any other reducing agent, could be used in the methods of the invention.

In addition, data in the Specification demonstrates that the method taught in Boshes does not result in the inactivation of ribonucleases in the manner described and claimed in the application. As described in the Specification, at page 13, line 28, to page 14, line 19, Boshes indicated that the addition of DTT (4 mM DTT at 4°C for 20 minutes) to a crude polyribosome preparation decreased the generation of monoribosomes by RNase A (10 mg/ml). However, when Applicants tested for inactivation of ribonucleases using the conditions suggested by Boshes, they found the Boshes method failed to inactivate ribonuclease. In these tests, RNase A (at 200 ng/ml, instead of the higher concentration of 10 mg/ml RNase A employed in Boshes) was treated with or without 4 mM DTT in Boshes' solution A at 4°C for 20 minutes. RNase A activity was then directly assayed by incubating mouse liver total RNA with the treated RNase A at 37°C for 60 minutes. The mouse RNA was then fractionated by electrophoresis in a 1% agarose formaldehyde gel to determine the level of RNA degradation. RNA degradation was evaluated by comparing the treated RNA with untreated mouse RNA.

As measured by the assay system described in the Specification, RNase A (200 ng/ml) treated with 4 mM DTT was not inactivated using the conditions set forth by Boshes. In fact, under Boshes's conditions, the RNase A treated with 4 mM DTT completely digested the RNA. Therefore, the conditions reported in Boshes are not sufficient to inhibit even 1/50 the

concentration of the RNase A employed in that paper in the manner claimed in the present invention. In contrast to RNase A treated using the Boshes method, RNase A treated at 60°C for 20 minutes in the presence of 20 mM DTT was completely inactivated, *i.e.*, the mouse RNA remained completely intact in the RNase A activity assay.

The Examiner urges that the Boshes reference provides motivation to substitute another disulfide-formation-capable reducing agent for DTT in the Murthy *et al.* process. However, as discussed above, the Murthy *et al.* reference does not teach or suggest inactivation of ribonucleases by DTT. Therefore, even if motivation existed in Boshes to substitute "another disulfide-formation-capable reducing agent for DTT," the combination of Murthy *et al.* and Boshes would not lead one to the claimed invention. Furthermore, there is no motivation to combine these references. Therefore, Murthy *et al.* combined with Boshes does not render the claimed invention obvious.

3. Cleland Combined with Murthy *et al.* Does Not Render the Claims Obvious.

Neither Cleland nor Murthy *et al.* contain the suggestion or motivation to combine these two references for any purpose. Further, even if such suggestion or motivation could be found, combining the references would not produce the methods claimed in the present application.

The Cleland reference reports that DTT is a superior protective agent for sulfhydryl groups, as it reduces disulfides and maintains them in the reduced state. *See* Cleland, *Biochem.* 3(4):480-482 (1964). Contrary to the Examiner's assertion that "Cleland discloses [ ] that there are numerous reducing agents with substantial equivalence to DTT, including DET, cysteine, and ß-mercaptoethanol," Cleland in fact indicates that DTT is much superior to these and other thiols that are used as protective reagents for sulfhydryl groups. Cleland, p. 480. Any discussion in Cleland regarding DTT or other agents as protective reagents for sulfhydryl groups is of no

relevance to the functioning of DTT or other reducing agents as RNase inactivators. Nowhere in Cleland is there any mention of the use of DTT, or any other reducing agent, and heat to inactivate ribonucleases. Therefore, the Cleland reference cannot remedy the failings of Murthy *et al.* or Boshes in this regard.

4. Prohibited Hindsight Provides the Only Rationale for Combining Murthy *et al.* with Other Art to Arrive at the Claimed Invention.

In attempting to combine the teachings of Murthy *et al.* (inactivation of ribonucleases with hPRI, not with a reducing agent) with Boshes (who indicated that the addition of DTT to a crude polyribosome preparation decreased the generation of monoribosomes by RNase A) or Cleland (who reports that DTT is a superior protective agent for sulfhydryl groups) to produce the methods claimed in Applicants' application, or any other reference cited, the Examiner is employing hindsight reconstruction of the invention, which is not permitted. "To imbue one of ordinary skill in the art with knowledge of the invention [ ], when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher." *W. L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540 (Fed.Cir. 1983).

The Federal Circuit has made it clear that "[t]here must be a reason or suggestion in the art for selecting the procedure used, other than the knowledge learned from the applicant's disclosure." *In re Dow Chemical Co.*, 837 F.2d 469 (Fed.Cir. 1988). But even if one somehow finds motivation to combine the references, neither Boshes nor Cleland remedy the failures of Murthy *et al.* with regard to the instant obviousness rejection.

From the above, it is apparent that the combination of Murthy *et al.*, Boshes, and Cleland completely fails to teach or suggest the methods of the claimed invention. In addition, there is no suggestion or motivation to combine the references. "When prior art references require a

selective combination to render obvious a subsequent invention, there must be some reason for the combination other than the hindsight gleaned from the invention itself...It is impermissible to use the claims as a frame and the prior art references as a mosaic to piece together a facsimile of the claimed invention." *Uniroyal Inc. v. Rudkin-Wiley Corp.*, 837 F.2d 1044 (Fed.Cir. 1988).

In view of the above, none of the rejected claims are obvious.

5. The Examiner Must Cite References or Provide Affidavits Supporting His Reliance On What is "Well-Known in the Art."

In rejecting the claims as obvious, the Examiner stated: "The substitution of another disulfide-formation-capable reducing agent for DTT in the Murthy *et al.* process, as motivated by the Boshes *et al.* reference, is *deemed* to be a variation on the Murthy *et al.* process which would have been *within the purview of the ordinary practitioner* seeking to optimize the Murthy process by selecting any one of the alternative reducing agents disclosed in the Boshes and Cleland references" (emphasis added). Action at page 8. There is no support for this assertion by the Examiner. Applicants traverse this assertion for reasons provided above, and, pursuant to section 2144.03 of the Manual of Patent Examining Procedure (MPEP), the Examiner must cite a reference or provide an affidavit supporting his position, or withdraw the grounds of rejection.

Immediately after the above quotation, the Examiner further stated: "The repetition of such a process as warranted is *deemed* to be a variation of the prior art specifically motivated by the variation in experimental conditions taught at p. 345, col. 2, line 4 et seq of Murthy *et al.*, and which is therefore *clearly within the purview of the ordinary practitioner* seeking to minimize RNAse activity in RNA samples" (emphasis added). Action at page 8. Again, there is no support for this assertion by the Examiner. As above, pursuant to MPEP § 2144.03, the

Examiner must cite a reference or provide an affidavit supporting his position, or withdraw the grounds of rejection.

For the reasons stated above, Applicants respectfully request that the rejections to claims 54-57 and 61-72 be withdrawn.

K. Claims 54-86 Are Not Obvious Over the Combination of Murthy *et al.* in View of Boshes and Cleland and in Further View of Chomczynski '515 and in Further View of Pasloske *et al.*

The Action rejects claims 84-86 as obvious over the combination of Murthy *et al.* in view of Boshes and Cleland, as discussed above, and in further view of Chomczynski '515 and of Pasloske *et al.*

This rejection includes those claims that are directed to methods and kits for the preparation of cDNA, which were excluded form the above rejection. Applicants have already set forth arguments against the combination of Murthy *et al.*, Boshes, and Cleland, and all of these arguments stand in regard to the present rejection. Applicants understand that the exclusion of claims 58-60 and 73-86 from the rejection premised on the combination of Murthy *et al.* in view of Boshes and Cleland alone indicates a belief of the Examiner that those references do not render obvious those claims. Therefore, the requisite motivation to combine these references with Chomczynski and/or Pasloske *et al.* in the context of claims directed to the isolation of cDNA cannot be found in Murthy *et al.*, Boshes, or Cleland. Further, this motivation cannot be found in Chomczynski and/or Pasloske *et al.*

1. Chomczynski Provides No Support for an Obviousness Rejection.

The Action states that:

> The use of a reducing agent by Chomczynski '515 is specified at column 4, line 24, thereby motivating the ordinary practitioner to elect alternatives to the thiol amine used by this reference as part of a modified isolation procedure devised to

improve on the prior art in the course of routine experimentation. The modification of Chomczynski '515 by substitution of alternative reducing agents is therefore clearly motivated by the primary '515 reference.

Applicants disagree.

The Chomczynski patent provides no teaching or motivation towards the presently claimed invention, because it teaches nothing about the use of a combination of a reducing agent and heat to inactivate ribonucleases. Rather, the method of Chomczynski relies on the use of chaotropic agents, usually guanidinium-based compounds in nucleic acid isolation processes. These chaotropic agents inhibit the activity of enzymes, including RNases. One of ordinary skill would not be motivated toward the present invention by Chomczynski, for at least two reasons." Iverson Declaration, paragraph 18, Appendix C.

First, the methods disclosed in Chomczynski, to the extent that they may disclose some reducing agents, do not disclose the use of a combination of a reducing agent and heat to treat cellular extracts in the manner of the claimed invention, and certainly would not motivate one of ordinary skill in the art toward the claimed invention, especially those aspects of the invention involving the preparation of cDNA from crude cell lysates. One of ordinary skill would understand that the chaotropic agents employed in Chomczynski are sufficient to inhibit any RNase that may be present in the Chomczynski preparations containing such chaotropic agents. "Therefore, in Chomczynski's compositions containing chaotropic agents, there is no need for an additional agent, such as a reducing agent, to inhibit ribonucleases." Iverson Declaration, paragraph 19, Appendix C. Additionally, in those embodiments described in Chomczynski where a reducing agent is present, there is no heating of the composition, and the procedures are preferred at room temperature or below. *Id.* Therefore, this aspect of the claimed invention is not taught by Chomczynski.

The combination of Chomczynski with the other cited art would result in inoperative embodiments of the invention, and this would be understood by those of skill. The failure of Chomczynski to heat its compositions would prevent any ability of the reducing agents to inactivate ribonucleases. Further, the chaotropic agents of Chomczynski would be expected by those of skill to inactivate any proteins added to the admixtures of the present claims, including reverse transcriptase as used in the claimed cDNA production procedures. Iverson Declaration, paragraph 19, Appendix C. For this reason, the methods of Chomczynski require washing of isolated RNA and DNA by centrifugation, pelleting, and resuspension of the nucleic acid in a solution that does not comprise the chaotropic agent, before that nucleic acid is used in a PCR or RT-PCR procedure. See specification, page 2, lines 27-33, and Iverson Declaration, paragraph 19, Appendix C. In view of the effects of chaotropic agents on enzymes, those of ordinary skill would not expect the chaotropic agent-containing lysates of Chomczynski to be useful in the methods of the present invention in which reverse transcriptase or another enzyme is added directly to an extract of a cell, and would not be motivated to combine the art in the manner suggested by the Action. Certainly, one of ordinary skill would not see a reasonable expectation of success for the claimed invention as a result of such a combination.

In contrast to the method of Chomczynski, the methods of the present invention do not require a chaotropic agent to inactivate ribonucleases. As shown in the specification, at page 17, line 1, to page 18, line 4, reducing agents do not effect enzymes such as reverse transcriptase and DNase I. Therefore, it is possible, in the context of the present invention, but not Chomczynski's method, to add enzymes directly to a cell extract containing the agent used to inactivate any ribonucleases and perform, for example, a reverse transcriptase reaction to produce cDNA. Iverson Declaration, paragraph 21, Appendix C.

2. The Examiner Must Cite References or Provide Affidavits Supporting His Reliance On What is "Well-Known in the Art."

The Action states, "The use of a reducing agent by Chomczynski '515 is specified at column 4, line 24, thereby motivating the ordinary practitioner to elect alternatives to the thiol amine used by this reference as part of a modified isolation procedure devised to improve on the prior art in the course of routine experimentation," Action at page 9. There is no support for the attribution of motivation or skill to the ordinary practitioner provided, and Applicants dispute this statement. Applicants traverse this assertion for reasons provided above, and, pursuant to section 2144.03 of the Manual of Patent Examining Procedure (MPEP), the Examiner is requested to cite a reference or provide an affidavit supporting his position as to "well-known prior art or knowledge on the part of one of ordinary skill," or withdraw the grounds of rejection.

Additionally, there is no support for the Action's statement, "The modification of Chomczynski '515 by substitution of alternative reducing agents is therefore clearly motivated by the primary '515 reference." Action at page 9. Applicants dispute this attribution of motivation or skill to the ordinary practitioner. Applicants traverse this assertion for reasons provided above, and, pursuant to section 2144.03 of the Manual of Patent Examining Procedure (MPEP), the Examiner is requested to cite a reference or provide an affidavit supporting his position as to "well-known prior art or knowledge on the part of one of ordinary skill," or withdraw the grounds of rejection.

3. Pasloske *et al.* Does Not Remedy the Defects of the Instant Rejection.

The Action cites Pasloske *et al.* in regard to this obviousness rejection, but does not give any details as to the manner in which the Examiner believes that the reference contributes to the rejection. Applicants assume that Pasloske *et al.* is cited for its teaching of Armored RNA ribonuclease resistant RNA standards, in the attempt to render obvious the claims that recite such

standards. In this regard, the Action fails to provide any indication of any motivation to combine Pasloske *et al.* with any of the other cited art, and certainly provides no reasonable expectation of success in this regard. Further, there is no such motivation and no such expectation of success. Therefore, this reference does not remedy the infirmities of this rejection.

In view of the above, all of the claims are allowable over the combination of Murthy *et al.* in view of Boshes and Cleland, as discussed above, and in further view of Chomczynski '515 and of Pasloske *et al.*

L. Claim 87 is Allowable over the Combination of Pasloske *et al.* and Mulder *et al.*

The Action rejected previous claim 87 as obvious over the combination of Pasloske *et al.* and Mulder *et al.* Applicants believe that this was because claim 87 was the only claim that did not recite the use or inclusion of a reducing agent. Without acquiescing in any way as to the Action's arguments concerning previous claim 87, Applicants have amended claim 87 such that it now recites a reducing agent. Therefore, this rejection is believed moot, and current claim 87 is allowable over all of the other cited art for reasons set forth above.

M. Conclusion

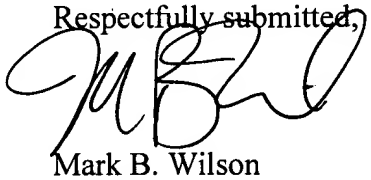
In view of the above remarks, Applicants respectfully submit that claims 1 and 14-30 are in condition for allowance. Reconsideration of the application and claims is courteously solicited. The Examiner is invited to contact the undersigned at (512) 536-3035 with any questions, comments or suggestions relating to the referenced patent application.

III. PETITION FOR EXTENSION OF TIME

Pursuant to 37 C.F.R. § 1.136(a), Applicants petition for an extension of time of three month to and including May 27, 2002, in which to respond to the Office Action dated November

27, 2001. Because Monday, May 27, 2002, is the Memorial Day Holiday in the District of Columbia, this petition renders the Response timely filed by deposition with the U.S. Postal Service on Tuesday, May 28, 2002, with an appropriate Certificate of Mailing. Pursuant to 37 C.F.R. § 1.17, a check in the amount of $460.00 is enclosed, which is the process fee for a three-month extension of time. If the check is inadvertently omitted, or should any additional fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason relating to the enclosed materials, or should an overpayment be included herein, the Commissioner is authorized to deduct or credit said fees from or to Fulbright & Jaworski Deposit Account No. 50-1212/10102270/MBW.

Respectfully submitted,



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Date: May 28, 2002

APPENDIX A
Amended Claims With Editing Indicia

54. (Amended) A method of treating an extract of a cell comprising:

(a) obtaining at least one cell;

(b) obtaining a reducing agent;

(c) preparing an admixture of an extract of the cell and the reducing agent; and

(d) heating the admixture.

56. (Amended) The method of claim [55] 54, wherein preparing an admixture of [the] an extract of the cell and the reducing agent comprises:

(a) first preparing [the] an extract of the cell; and

(b) then mixing the extract with the reducing agent.

57. (Amended) The method of claim [55] 54, wherein preparing an admixture of [the] an extract of the cell and the reducing agent comprises:

(a) first mixing the cell and the reducing agent; and

(b) then preparing [the] an extract [from] of the cell in the presence of the reducing agent.

73. (Amended) A method for producing cDNA from one or more cells comprising:

(a) obtaining at least one cell;

(b) obtaining a reducing agent;

(c) preparing an admixture of an extract of the cell and the reducing agent;

(d) heating the admixture; and

(e) incubating the admixture with reverse transcriptase under conditions to allow reverse transcription.

75. (Amended) The method of claim [74] 73, wherein preparing an admixture of [the] an extract of the cell and the reducing agent comprises:

(a) first preparing [the] an extract of the cell; and

(b) then mixing the extract with the reducing agent.

76. (Amended) The method of claim [74] 73, wherein preparing an admixture of [the] an extract of the cell and the reducing agent comprises:

(a) first mixing the cell and the reducing agent; and

(b) then preparing [the] an extract [from] of the cell in the presence of the reducing agent.

80. (Amended) A kit for producing cDNA from a cell, comprising, in one or more suitable containers:

(a) a buffer; and

(b) a reducing agent.

86. (Amended) The kit of claim 80, further comprising [Armored RNA®] a ribonuclease resistant RNA standard.

87. (Amended) A kit for producing cDNA from a cell comprising, in one or more suitable container(s):

(a) a cell lysis buffer;

(b) a deoxyribonuclease;

(c) an RNase inhibitor;

(d) a reverse transcription buffer;

(e) reverse transcriptase;

(f) dNTPs; [and

(g) Armored RNA®.] a reducing agent; and

(h) a ribonuclease resistant RNA standard.

88. (New) The method of claim 54, wherein the admixture is heated to a temperature that would result in the inactivation of RNase A, if any, present in the admixture.

89. (New) The method of claim 54, wherein the admixture is heated to a temperature that would result in the inactivation of RNase 1, if any, present in the admixture.

90. (New) The method of claim 54, wherein the admixture is heated to a temperature that would result in the inactivation of RNase T1, if any, present in the admixture..

91. (New) The method of claim 54, wherein the admixture initially comprises at least one ribonuclease which is inactivated by the combination of the reducing agent and heating.

92. (New) The method of claim 91, wherein the at least one ribonuclease is further defined as RNase A, RNase 1, or RNase T1.

93. (New) The method of claim 91, wherein the at least one ribonuclease is RNase A.

94. (New) The method of claim 91, wherein the at least one ribonuclease is RNase T1.

95. (New) The method of claim 91, wherein the at least one ribonuclease is RNase 1.

96. (New) The method of claim 54, wherein the admixture comprises at least one ribonuclease which was comprised in the cell and which is inactivated by the combination of the reducing agent and heating.

97. (New) The method of claim 54, wherein the reducing agent is a thiol-containing reducing agent.

98. (New) The method of claim 73, wherein the admixture initially comprises at least one ribonuclease which is inactivated by the combination of the reducing agent and heating.

99. (New) The method of claim 91, wherein the at least one ribonuclease is further defined as RNase A, RNase 1, or RNase T1.

100. (New) The method of claim 91, wherein the at least one ribonuclease is RNase A.

101. (New) The method of claim 91, wherein the at least one ribonuclease is RNase T1.

102. (New) The method of claim 91, wherein the at least one ribonuclease is RNase 1.

103. (New) The method of claim 91, wherein the admixture comprises at least one ribonuclease which was comprised in the cell and which is inactivated by the combination of the reducing agent and heating.

104. (New) The method of claim 79, wherein the reducing agent is DTT.

105. (New) The method of claim 104, wherein said the final concentration of the DTT is between 1 and 200 mM in the admixture.

106. (New) The method of claim 105, wherein the final concentration of DTT is 20 mM in the admixture.

107. (New) The method of claim 79, wherein said reducing agent is β-mercaptoethanol.

108. (New) The method of claim 107, wherein the final concentration of β-mercaptoethanol is between 1 and 200 mM in the admixture.

109. (New) The method of claim 79, wherein said reducing agent is cysteine.

110. (New) The method of claim 109, wherein the final concentration of cysteine is between 1 and 200 mM in the admixture.

111. (New) The method of claim 73, wherein the reducing agent is a thiol-containing reducing agent.

112. (New) The method of claim 73, wherein the reducing agent is comprised in a buffer composition prior to preparation of the admixture.

113. (New) The method of claim 73, wherein the admixture is heated to at least 37°C.

114. (New) The method of claim 113, wherein the admixture is heated to at least 60°C.

115. (New) The method of claim 73, wherein the admixture is heated to a temperature that would result in the inactivation of RNase A, if any, present in the admixture.

116. (New) The method of claim 73, wherein the admixture is heated to a temperature that would result in the inactivation of RNase 1, if any, present in the admixture.

117. (New) The method of claim 73, wherein the admixture is heated to a temperature that would result in the inactivation of RNase T1, if any, present in the admixture.

118. (New) The method of claim 73, wherein the admixture is heated for at least 4 minutes.

119. (New) The method of claim 87, wherein the reducing agent is comprised in the lysis buffer.

120. (New) The method of claim 73, further defined as a method of preparing cDNA from a cellular extract without RNA purification.

APPENDIX B
Pending Claims

54. A method of treating an extract of a cell comprising:

(a) obtaining at least one cell;

(b) obtaining a reducing agent;

(c) preparing an admixture of an extract of the cell and the reducing agent; and

(d) heating the admixture.

56. The method of claim 54, wherein preparing an admixture of an extract of the cell and the reducing agent comprises:

(a) first preparing an extract of the cell; and

(b) then mixing the extract with the reducing agent.

57. The method of claim 54, wherein preparing an admixture of an extract of the cell and the reducing agent comprises:

(a) first mixing the cell and the reducing agent; and

(b) then preparing an extract of the cell in the presence of the reducing agent.

58. The method of claim 54, further defined as a method for producing cDNA from one or more cells and further comprising incubating the admixture with reverse transcriptase under conditions to allow reverse transcription.

59. The method of claim 58, further comprising amplifying the products of the reverse transcription.

60. The method of claim 58, further comprising incubating said admixture with a deoxyribonuclease prior to the reverse transcription reaction.

61. The method of claim 54, wherein the said reducing agent is DTT, β-mercaptoethanol, cysteine, or dithioerythritol.

62. The method of claim 54, wherein the reducing agent is DTT.

63. The method of claim 54, wherein said the final concentration of the DTT is between 1 and 200 mM in the admixture.

64. The method of claim 63, wherein the final concentration of DTT is 20 mM in the admixture.

65. The method of claim 54, wherein said reducing agent is β-mercaptoethanol.

66. The method of claim 65, wherein the final concentration of β-mercaptoethanol is between 1 and 200 mM in the admixture.

67. The method of claim 54, wherein said reducing agent is cysteine.

68. The method of claim 67, wherein the final concentration of cysteine is between 1 and 200 mM in the admixture.

69. The method of claim 54, wherein the reducing agent is comprised in a buffer composition prior to admixing.

70. The method of claim 54, wherein the admixture is heated to at least 37°C.

71. The method of claim 70, wherein the admixture is heated to at least 60°C.

72. The method of claim 54, wherein the admixture is heated for at least 4 minutes.

73. A method for producing cDNA from one or more cells comprising:

(a) obtaining at least one cell;

(b) obtaining a reducing agent;

(c) preparing an admixture of an extract of the cell and the reducing agent;

(d) heating the admixture; and

(e) incubating the admixture with reverse transcriptase under conditions to allow reverse transcription.

75. The method of claim 73, wherein preparing an admixture of an extract of the cell and the reducing agent comprises:

(a) first preparing an extract of the cell; and

(b) then mixing the extract with the reducing agent.

76. The method of claim 73, wherein preparing an admixture of an extract of the cell and the reducing agent comprises:

(a) first mixing the cell and the reducing agent; and

(b) then preparing an extract of the cell in the presence of the reducing agent.

77. The method of claim 73, further comprising amplifying the products of the reverse transcription.

78. The method of claim 73, further comprising incubating said admixture with a deoxyribonuclease prior to the reverse transcription reaction.

79. The method of claim 73, wherein the said reducing agent is DTT, β-mercaptoethanol, cysteine, or dithioerythritol.

80. A kit for producing cDNA from a cell, comprising, in one or more suitable containers:

(a) a buffer; and

(b) a reducing agent.

81. The kit of claim 80, wherein the buffer and the reducing agent are comprised in the same container.

82. The kit of claim 80, further comprising, in one or more container(s):

(c) a reverse transcription buffer

(d) a reverse transcriptase; and

(e) a dNTP mix.

83. The kit of claim 80, further comprising a deoxyribonuclease.

84. The kit of claim 80, wherein said reducing agent is DTT.

85. The kit of claim 80, further comprising an RNase inhibitor.

86. The kit of claim 80, further comprising a ribonuclease resistant RNA standard.

87. A kit for producing cDNA from a cell comprising, in one or more suitable container(s):

(a) a cell lysis buffer;

(b) a deoxyribonuclease;

(c) an RNase inhibitor;

(d) a reverse transcription buffer;

(e) reverse transcriptase;

(f) dNTPs;

(g) a reducing agent; and

(h) a ribonuclease resistant RNA standard.

88. The method of claim 54, wherein the admixture is heated to a temperature that would result in the inactivation of RNase A, if any, present in the admixture.

89. The method of claim 54, wherein the admixture is heated to a temperature that would result in the inactivation of RNase 1, if any, present in the admixture.

90. The method of claim 54, wherein the admixture is heated to a temperature that would result in the inactivation of RNase T1, if any, present in the admixture.

91. The method of claim 54, wherein the admixture initially comprises at least one ribonuclease which is inactivated by the combination of the reducing agent and heating.

92. The method of claim 91, wherein the at least one ribonuclease is further defined as RNase A, RNase 1, or RNase T1.

93. The method of claim 91, wherein the at least one ribonuclease is RNase A.

94. The method of claim 91, wherein the at least one ribonuclease is RNase T1.

95. The method of claim 91, wherein the at least one ribonuclease is RNase 1.

96. The method of claim 54, wherein the admixture comprises at least one ribonuclease which was comprised in the cell and which is inactivated by the combination of the reducing agent and heating.

97. The method of claim 54, wherein the reducing agent is a thiol-containing reducing agent.

98. The method of claim 73, wherein the admixture initially comprises at least one ribonuclease which is inactivated by the combination of the reducing agent and heating.

99. The method of claim 91, wherein the at least one ribonuclease is further defined as RNase A, RNase 1, or RNase T1.

100. The method of claim 91, wherein the at least one ribonuclease is RNase A.

101. The method of claim 91, wherein the at least one ribonuclease is RNase T1.

102. The method of claim 91, wherein the at least one ribonuclease is RNase 1.

103. The method of claim 91, wherein the admixture comprises at least one ribonuclease which was comprised in the cell and which is inactivated by the combination of the reducing agent and heating.

104. The method of claim 79, wherein the reducing agent is DTT.

105. The method of claim 104, wherein said the final concentration of the DTT is between 1 and 200 mM in the admixture.

106. The method of claim 105, wherein the final concentration of DTT is 20 mM in the admixture.

107. The method of claim 79, wherein said reducing agent is β-mercaptoethanol.

108. The method of claim 107, wherein the final concentration of β-mercaptoethanol is between 1 and 200 mM in the admixture.

109. The method of claim 79, wherein said reducing agent is cysteine.

110. The method of claim 109, wherein the final concentration of cysteine is between 1 and 200 mM in the admixture.

111. The method of claim 73, wherein the reducing agent is a thiol-containing reducing agent.

112. The method of claim 73, wherein the reducing agent is comprised in a buffer composition prior to preparation of the admixture.

113. The method of claim 73, wherein the admixture is heated to at least 37°C.

114. The method of claim 113, wherein the admixture is heated to at least 60°C.

115. The method of claim 73, wherein the admixture is heated to a temperature that would result in the inactivation of RNase A, if any, present in the admixture.

116. The method of claim 73, wherein the admixture is heated to a temperature that would result in the inactivation of RNase 1, if any, present in the admixture.

117 The method of claim 73, wherein the admixture is heated to a temperature that would result in the inactivation of RNase T1, if any, present in the admixture.

118. The method of claim 73, wherein the admixture is heated for at least 4 minutes.

119. The method of claim 87, wherein the reducing agent is comprised in the lysis buffer.

120. The method of claim 73, further defined as a method of preparing cDNA from a cellular extract without RNA purification.